CHAPTER 2

Literature review

Essential oils are complex mixtures of volatile secondary metabolites in aromatic plants derived from fruits (e.g. bergamot, orange, lemon), flowers (e.g. rose, jasmine, lavender), leave (e.g. mint, basil), bark (e.g. cinnamon, cassia), and seeds (e.g. fennel, coriander, caraway). The majority of essential oils are a source of aroma compounds and they are also used as fragrances in perfumery as well as in cosmetics, food, and beverages industry.[8] Various essential oils have been shown biological activity including antioxidant, anti-inflammatory, insecticidal, antiviral, antibacterial, antifungal, and anti-cancerogenic properties.[12,13]

2.1 Chemical compositions of essential oils [14,15]

Generally, the main constituents of essential oils are hydrocarbons and oxygenated compounds classified by structural formulas as terpenes, alcohols, phenols, ethers, aldehydes, and ketones. These compounds are responsible for the fragrant and biological properties of aromatic and medicinal plants.

2.1.1 Terpenes

Terpenes are the chemical substances having fundamental repeating five-carbon isoprene units (Figure 2.1). These compounds are defined as a unique group of hydrocarbon based natural products. Commonly, terpenes can be subdivided into hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), triterpenes (C_{30}) and tetraterpenes (C_{40}). However, the terpenes, which are mainly found in the essential oils, are usually monoterpenes and sesquiterpenes.

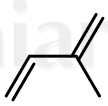


Figure 2.1 The carbon skeleton of isoprene unit

Monoterpenes are usually possible to detect the presence of two isoprene units that the molecular formula is $C_{10}H_{16}$ such as α-pinene, myrcene, camphene, α-thujene, ocimene, and α-phellandrene whereas sesquiterpenes consist of three isoprene units and conform to the molecular formula of $C_{15}H_{24}$ such as α-zingiberene, (*E*)-caryophyllene, curcumene, β-farnesene, α-humulene, and germacrene D. The structures of some hydrocarbon compounds are shown in Figure 2.2.

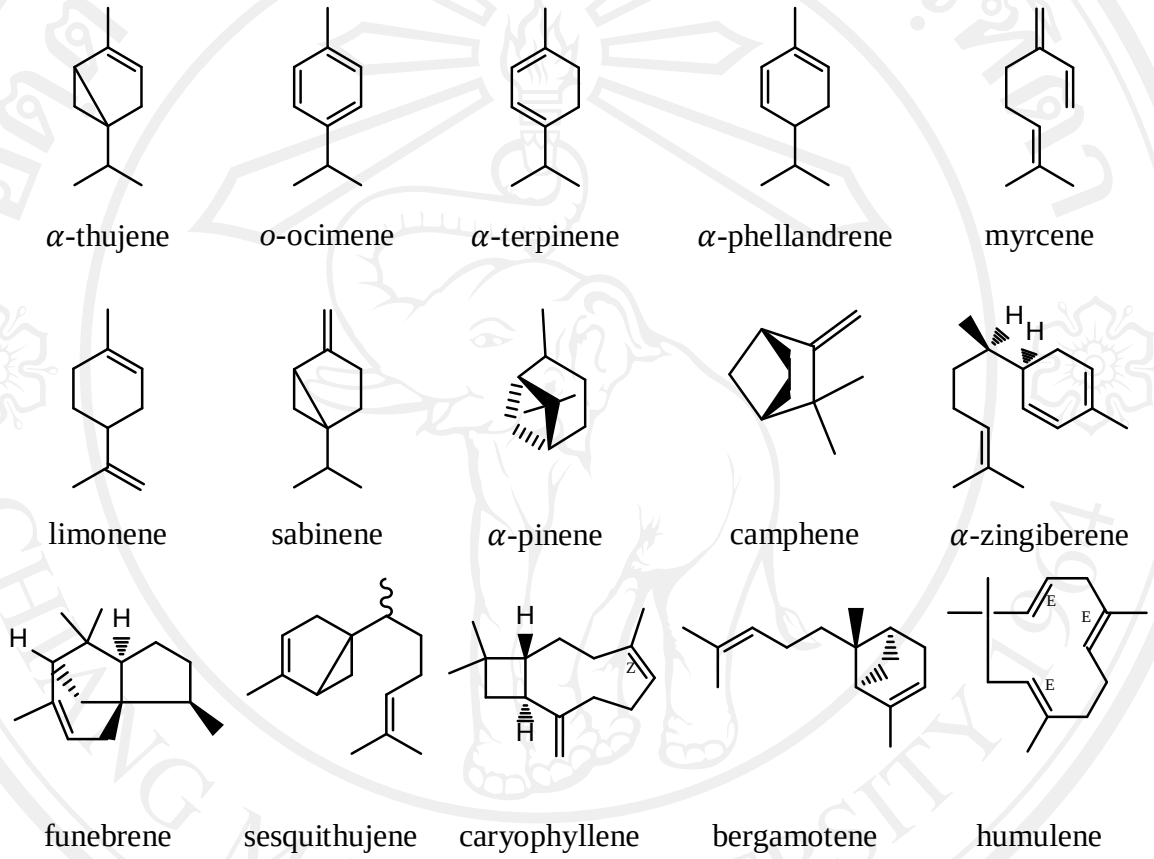


Figure 2.2 Some structures of hydrocarbon compounds

2.1.2 Oxygenated derivatives

In addition, the containing oxygen of terpenes is called terpenoids or oxygenated compounds. The characteristic odor of many essential oils is representative from the oxygenated derivatives that the main functional groups are alcohols, ethers, aldehydes, and ketones. For example, alcohols consist of a hydroxyl group (-OH) attached to one of the carbons in an aliphatic chain by displacing one of the hydrogen molecules such as geraniol, linalool, citronellol, lavandulol, and nerol.

While phenols, which is a kind of alcohols, consist of hydroxyl group attached to a carbon in the aromatic ring such as thymol, and carvacrol. Aldehyde derivatives contain aldehyde radical (-CHO) in the basic structure such as citral, cinnamic aldehyde, citronellal, geranial, neral and cuminal. Moreover, ketones consist of a carbonyl group attached to a carbon on a chain structure such as menthone, carvone, and pulegone. The structures of some oxygenated compounds are shown in Figure 2.3. In addition, the chemical compounds of essential oil have been associated with various therapeutic activities. The classification of the component and their bioactivities are shown in Table 2.1.

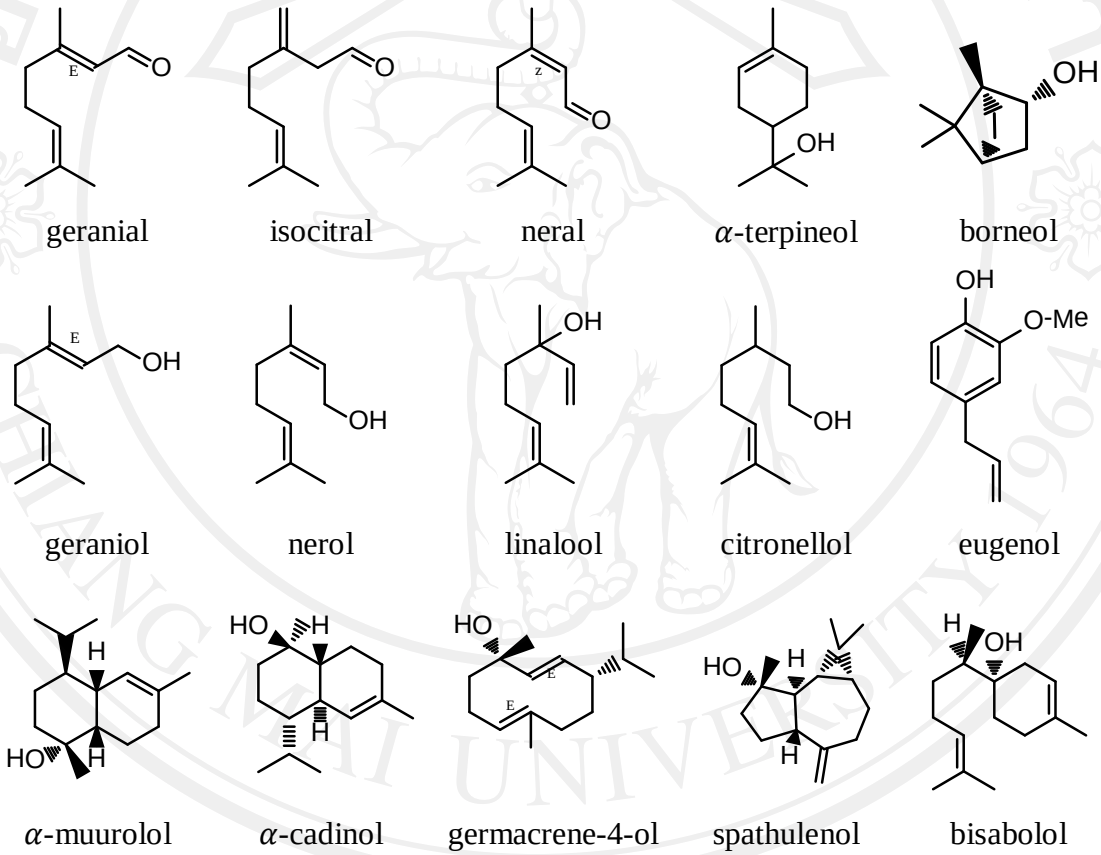


Figure 2.3 Some structures of oxygenated compounds

Table 2.1 Different classes of essential oils compounds and their bioactivities [15]

Class of compounds	Example	Bioactivities
Hydrocarbons		
Terpenes	limonene, myrcene, pinene, sabinene, cymene, myrcene, phellandrene	stimulant, antiviral, antitumour, decongestant, antibacterial, hepatoprotective
Oxygenated derivatives		
Esters	linalyl acetate, geraniol acetate, eugenol acetate, bornyl acetate	spasmolytic, sedative, antifungal, anaesthetic, anti-inflammatory
Alcohols	linalol, menthol, borneol, santalol, nerol, citronellol, geraniol	antimicrobial, antiseptic, balancing, spasmolytic, anti-inflammatory
Phenols	thymol, eugenol, carvacrol, chavicol	antimicrobial, spasmolytic, anaesthetic, irritant, immune stimulating
Aldehydes	citral, myrtenal, citronellal, benzaldehyde cinnamaldehyde,	antiviral, antimicrobial, tonic, vasodilators, hypotensive, calming, antipyretic, sedative, spasmolytic
Ketones	carvone, menthone, pulegone, fenchone, camphor, thujone,	mucolytic, cell regenerating, sedative, antiviral, neurotoxic, analgesic, digestive, spasmolytic
Oxides	bisabolone oxide, linalool oxide, sclareol oxide,	anti-inflammatory, expectorant, stimulant

2.2 Extraction of essential oils [14,16]

Essential oils are used in a wide variety of consumer goods such as detergents, soaps, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, and insecticides. Production of essential oil is an essential basis to improve the yield and quality of the oils. There are several techniques to extract essential oil from plants depending on their substance, stability and concentration. Regularly, expression, solvent extraction, and distillation are the most traditional and commonly used methods.

2.2.1 Expression

Expression is a cold pressed method of extraction, which is mostly used in the extraction of citrus essential oils such as bergamot, lemon, lime, orange oils. Many components of these essential oils are delicate and suffer significantly from heat degradation when exposed to steam distillation. In term of expression is referred to any physical process which is absently used a heat source. The essential oil glands in the peel are crushed or broken to release the oils. The oils extracted contain water, but this water can be evaporated, remaining just the essential oils.

2.2.2 Solvent extraction

Solvent extraction is a common process to remove the oil from plants in large-scale operations at low temperature using an organic solvent such as hexane and petroleum ether. The solvent extraction is suitable for the plant contains very little essential oil. The solvent penetrates the plant material and dissolves the aromatic compounds, consisting of volatile oils, waxes, fatty acids, and coloring matter. After the solvent is distilled off at a low temperature under partial vacuum, the remaining constituents make up the concrete. Subsequently, alcohol is used to extract the volatile oil from the other constituents. The alcohol is eliminated by distillation and an absolute, which is considered a true aromatic treasure, is obtained. The extraction is more costly process than water or steam distillation. Moreover, many solvents are highly inflammable and harmful to the environment.

2.2.3 Enfleurage

Enfleurage is one of the oldest techniques applied to capture the true odor of the most delicate flowers. The enfleurage is the process of transferring the volatile compounds to a fixed oil or fat spread out on a glass plate by absorption. The flowers are placed on the fat and left to release their oils for several days. The process is repeated several more times with fresh flowers being added to the plates until the fat on the plates is completely saturated with the aromatic oils of the flowers.[17] The product is called pomade which is used directly in cosmetics. However, the pomade is usually dissolved by alcohol to separate the essential oil from the fat and then the alcohol is evaporated to leave the pure aromatic oil of the flower.

2.2.4 Distillation

Distillation process is the most widely used for the extraction of essential oils. The important forms of distillation, which are applied to produce the essential oils, consist of water distillation, steam distillation and hydrodiffusion.

Water distillation

In water distillation, the plant material is completely immersed in water, which is boiled by applying heat. Due to the influence of hot water, the essential oil is freed from the oil glands in the plant tissue. The vapor mixture of water and oil is condensed by indirect cooling with water. The oil, which is immiscible with water, can be separated. The main characteristic of this process is directly contacted between boiling water and plant material.

Wet steam distillation

Wet steam distillation, plant Material is supported on a perforated grid or screen inserted some distance above the bottom of the still that the equipment used is similar with water distillated equipment. The lower part of the still is filled with water. The water is heated and then the saturated and wet low pressure steam rises through plant material. The vapor mixture of water and oil is condensed and collected like water distillation. In this process, all parts of the plant are uniformly contacted by steam for obtaining high yield of the essential oil.

Dry steam distillation

Dry steam distillation, the steam is generated outside the still in a satellite steam generator. The plant material is placed on a perforated grid above the steam inlet. High pressure steam, which is produced in the generator, is forced through the material to be distilled. The advantages are that the amount of steam can be readily controlled. Moreover, the plant material is heated no higher than 100°C. Consequently, it should not undergo thermal degradation.

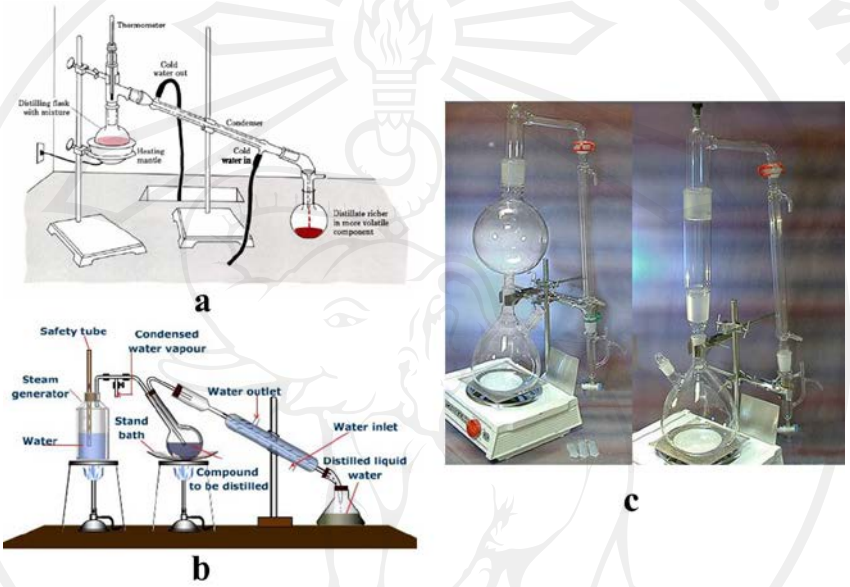


Figure 2.4 hydrodistillation (a), steam distillation (b), steam distillation and hydrodistillation apparatus (c)

Hydrodiffusion

Hydrodiffusion is the process used to extract the essential oils at low-temperature and low-pressure steam. The volatile components are extracted mainly by osmosis. Steam is fed from the top of the still and the steam moves downwards through the plant materials by gravity. At the temperature of boiling water, a part of volatile oil dissolves in the water present within the glands. This oil-water solution permeates by osmosis the swollen membranes and finally reaches the outer surface. Then, the steam and volatile compounds flow through a condenser placed at the bottom of the still and are collected in an oil separator. Hydrodiffusion has shown excellent results under experimental conditions such as short distillation times, low steam consumption, high yields of high-quality oil and absence of high temperatures.

2.3 Gas chromatography-mass spectrometry (GC-MS) [18,19]

Gas chromatography-mass spectrometry (GC-MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS) as show in Figure 2.5. Complex mixtures of chemicals can be separated, identified and quantified by this technique. GC-MS makes it ideal for the analysis of the hundreds of relatively low molecular weight compounds found in environmental materials. In order for a compound to be analyzed by the technique, it must be sufficiently volatile and thermally stable. Samples are usually analyzed as organic solutions. The materials, which are interested such as soils, sediments, tissues, and plants, need to be extracted with organic solvent and the extract subjected to various wet chemical techniques before analysis.

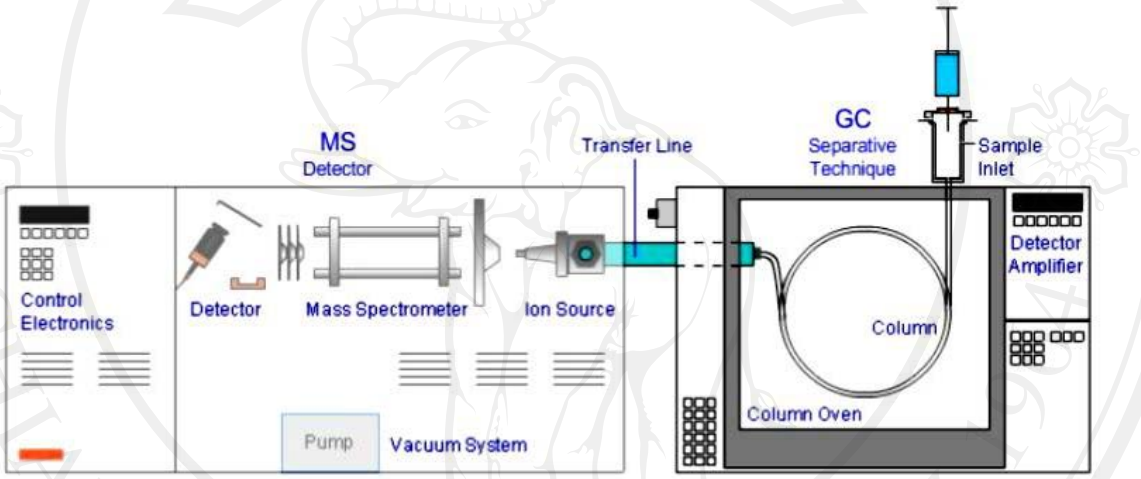


Figure 2.5 Gas chromatography-mass spectrometry diagram

The sample solution is injected into the GC inlet where it is vaporized and swept onto a chromatographic column by the carrier gas (usually helium, He). The sample flows through the column and the compounds are separated by virtue of their relative interaction with the stationary phase of the column and the carrier gas. The latter part of the column passes through a heated transfer line and ends at the entrance to ion source where compounds eluting from the column are converted to ions. Two potential methods exist for ion production. The most frequently used method is electron ionisation (EI) and the occasionally used alternative is chemical ionisation (CI).

Electron ionisation (EI)

A beam of electrons ionise the sample molecules resulting in the loss of one electron. A molecule with one electron missing is called the molecular ion and is represented by a radical cation ($M^{+\cdot}$) (Equation 2.1). When the resulting peak from this ion is seen in a mass spectrum, it gives the molecular weight of the compound. Due to the large amount of energy imparted to the molecular ion it usually fragments producing further smaller ions with characteristic relative abundances that provide a fingerprint for that molecular structure. This information may be then used to identify compounds of interest and help elucidate the structure of unknown components of mixtures.

$$M + e^- \longrightarrow M^{+\cdot} + 2e^-$$
$$M^{+\cdot} \longrightarrow F^+ + N\cdot \qquad \text{(Equation 2.1)}$$

Chemical ionisation (CI)

CI begins with the ionisation of methane (or another suitable gas), creating a radical which in turn will ionise the sample molecule to produce $[M+H]^+$ molecular ions (Equation 2.2). CI is a less energetic way of ionising a molecule hence less fragmentation occurs with CI than with EI. Therefore, CI yields less information about the detailed structure of the molecule, but does yield the molecular ion. Sometimes the molecular ion cannot be detected using EI, hence the two methods complement one another. Once ionised a small positive is used to repel the ions out of the ionisation chamber.

$$CH_4 + e^-\cdot \longrightarrow CH_4^{+\cdot} + 2e^-$$
$$CH_4^{+\cdot} + CH_4 \longrightarrow CH_5^+ + CH_3^+$$
$$CH_5^+ + M \longrightarrow CH_4 + MH^+ \qquad \text{(Equation 2.2)}$$

The mass analyser separates the positively charged ions according to various mass related properties depending upon the analyser used. Several types of mass analyser exist: quadrupoles (Figure. 2.6), ion traps, magnetic sector, time-of-flight, radio frequency, cyclotron resonance and focusing to name a few. The most common are quadrupoles and ion traps. After the ions are separated they enter a detector the output from which is amplified to boost the signal. The detector sends information to a computer that records all of the data produced, converts the electrical impulses into visual displays and hard copy displays. In addition, the computer also controls the operation of the mass spectrometer.

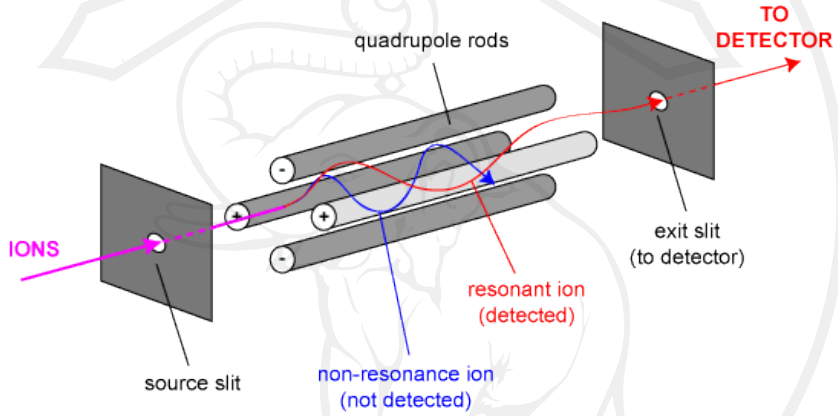


Figure 2.6 A schematic of a quadrupole analyser

2.3.1 Qualitative analysis [18,20,21]

Modern capillary GC is characterized by very good precision in retention time which is used for qualitative Analysis. However, retention time depend on many operational factors such as temperature, column length, diameter, film thickness, and carrier gas velocity and pressure. Therefore, the retention index allows the use for peak identification.

Retention index is developed by Kovats Index for isothermal conditions. The retention index is a concept used in gas chromatography to convert retention times into system-independent constants based on a series of standards such as straight chain hydrocarbons. The retention index value of a substance is defined according to Equation 2.3.

$$RI_{(X)} = 100Z + 100\Delta Z\left[\frac{(logRT_{(X)} - logRT_{(Z)})}{(logRT_{(Z+1)} - logRT_{(Z)})}\right] \quad \text{(Equation 2.3)}$$

Where: $RT_{(Z)} \leq RT_{(X)} \leq RT_{(Z+1)}$

RI = retention index

RT = retention time

X = the compound of interest

Z = number of carbon atom of *n*-alkanes eluting closely before compound X

$Z+1$ = number of carbon atom $(Z+1)$ of *n*-alkanes eluting closely after compound X

However, For mixtures of wide boiling point range the determination of retention indices under isothermal conditions would be time consuming and unnecessarily restrictive. The temperature programmed retention index (I_{TPRI}) is developed by Van den Dool and Kratz. In temperature-programmed can be calculated using direct numbers instead of their logarithm as shown in Equation 2.4.

$$RI_{(X)} = 100Z + 100\Delta Z\left[\frac{(RT_{(X)} - RT_{(Z)})}{(RT_{(Z+1)} - RT_{(Z)})}\right] \quad \text{(Equation 2.4)}$$

Where: $RT_{(Z)} \leq RT_{(X)} \leq RT_{(Z+1)}$

RI = retention index

RT = retention time

X = the compound of interest

Z = number of carbon atom of *n*-alkanes eluting closely before compound X

$Z+1$ = number of carbon atom $(Z+1)$ of *n*-alkanes eluting closely after compound X

The retention index of any substance is equivalent to 100-times the carbon number of hypothetical n-alkanes with the same adjusted retention time. The Kovats retention index is a useful tool for the comparison of retention data obtained by various conditions, as it is nearly independent on many of the parameters and conditions of the gas chromatographic analysis.

2.4 Acne

Skin is the largest organ of the human body. Many people, especially women, have always taken very good care of it. People suffer from many skin problems such as wrinkles, photoaging, mottled pigmentation, inflammation, and skin cancer. [22] One of common skin disease, which is concerned by several people, is acne. Acne is most common during adolescence, affecting more than 85% of teenagers, and frequently continues into adulthood. The cause in adolescence is generally an increase in male sex hormones, which people of both genders accrue during puberty.[23] Acne vulgaris also cause by changes in the pilosebaceous units, skin structures consisting of a hair follicle and its associated sebaceous gland via androgen stimulation.[24] The characterizations of acne consist of non-inflammation (follicular papules or comedones) and inflammation (papules, pustules, and nodules) that a severe forms of acne is shown on the skin including the face, the upper part of the chest, and the back.

2.4.1 Bacteria causing skin diseases

Skin is a barrier between the internal organs and the environment. Bacteria are widely distributed in nature and are normally present on the skin, in the mouth and nasal passages, and the intestines of human and animals. Skin disease can occur on the skin surface or deeper within the skin tissue. Bacteria that cause infections of the skin are as follows.

***Propionibacterium acnes* (*P. acnes*)** [5,25]

Propionibacterium acnes has been implicated in the pathogenesis of acne. It is initially believed to be the direct cause of the disease. *P. acnes*, an anaerobic gram-positive bacterium that normally resides in the pilosebaceous unit of the skin. One nutritional requirement of this bacterium is glycerol, obtained through lipolysis of the triglycerides in the sebum, which releases free fatty acids as byproducts. The lipolysis process is shown in Figure 2.7. *P. acnes* also releases various chemotactic products, which attract neutrophils to the area. These neutrophils secrete hydrolytic enzymes that cause secondary damage to the follicular wall. The irritating free fatty acids and other bacterial enzymes can leak into the dermis, creating intense inflammation.

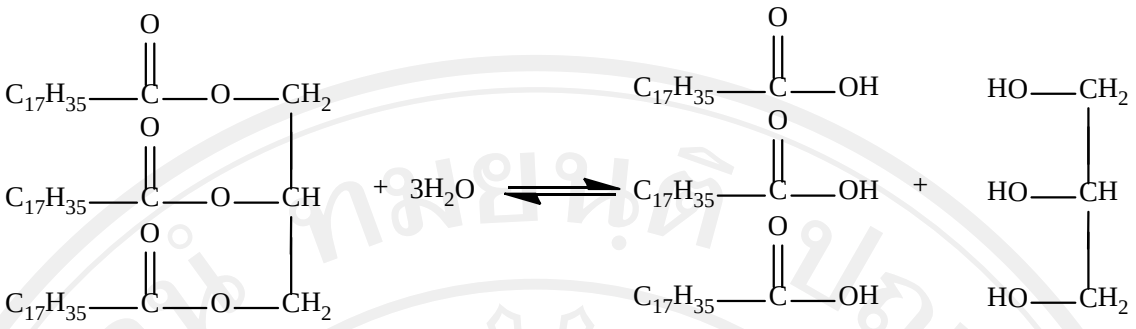


Figure 2.7 Lipolysis of the triglycerides in the sebum

***Staphylococcus aureus* (*S. aureus*)** [26]

Staphylococcus aureus is an aerobic gram-positive bacterium. *S. aureus* produce a variety of infections that involve any and all tissues of the human body. The disease states are characterized by pus formation. This bacterium is also a common cause of the inflammation on acne.

***Staphylococcus epidermidis* (*S. epidermidis*)** [27]

Staphylococcus epidermidis is an aerobic gram-positive bacterium. It typically lives on the skin and mucosa membranes of human and animals. This bacterium cause most infections on catheters and implants, also considered an opportunist pathogen on body infections, including acne inflammation. *S. epidermidis* may be isolated from stitch abscesses and other skin wounds.

2.4.2 Plants used for the treatment of acne vulgaris [23]

Several plants and plant-based preparations are used for the treatment of acne. Some of them are discussed below:

Asparagus

The flesh roots and seeds of Asparagus are important part of the medical therapy. Roots contain inulin, glycoside bitter principles (officinalisins I and II), β-sitosterol, steroidal glycosides and asparagusic acid. The plant extracts used as topical facial cleanser against acne.

Calendula

The flowers have been used for the treatment of various skin diseases and reduce inflammation. The herb contains flavonoids (quercetin), saponins (arvenoside A), triterpinoid, and essential oils. The flavonoids are used for the healing purpose of acne and many other skin diseases.

Rose

The most common flower is one of the most useful acne healers. The aqueous extract of the petals of the Rosa species are used for the daily care of the skin. The rose water is also effective against acne and black heads. The main constituents are tanninseugeniin, pentagalloyl, rugosal, phenylethyl alcohol and essential oil.

Tea tree

The leaves of this plant are the source of valuable therapeutic oil. The main constituent in tea tree essential oil is terpin-4-ol, present in concentrations of 40% or more. Tea tree oil is effective against a wide range of organisms including twenty seven of the 32 strains of *P. acnes*. Tea tree oil does not irritate to the skin and good penetration.

Thyme

The leaves are used for the treatment of cuts, burns, and acne in the area of the face, neck, throat or forehead. The main constituents are carvacrol, *p*-cymene, thymol, thymol acetate and apigenin.

Turmeric

The major chemical constituents of Turmeric include curcuminoids (the yellow colouring principle), curcumin, and essential oil with high content of bisatiolane derivatives. Turmeric exhibits remarkable anti-inflammatory activity attributed to the curcumin.

2.5 Antibacterial activity of essential oils

Nowadays, people are interested in using skin care products with made from natural constituents. The product for acne treatment is one which is developed. Consequently, the investigations of new natural bioactive compounds have been attracted in recent years and the essential oil is one of interesting compound. In that regards, the antibacterial activity of essential oil has been observed.

Ten essential oil, including mint, ginger, lemon, grapefruit, jasmine, lavender, chamomile, thyme, rose, and cinnamon were tested for their antibacterial activities towards *P. acnes*. The result indicated that thyme, cinnamon, and rose essential oils exhibited the best antibacterial activities towards *P. acnes*, with MIC of 0.016, 0.016, and 0.031% (v/v), respectively.[28] Moreover, six essential oil, including citrus, olive, ajwain, almond, bavchi, and neem oils were investigated for their antibacterial activities. These essential oils have been shown very high susceptibility against bacterial strains consisting of *Lactobacillus acidophilus*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *Escherichia coli*. From the results, the citrus, olive, ajwain, almond, bavchi, and neem oils could inhibit the growth of *S. aureus* at the MIC 2.0, 0.25, 4.0, 1.0, 0.125, and 4.0 µl/ml, respectively.[29]

The essential oil of *Citrus obovoides*, which was dominated by γ-terpinene (6.6%), limonene (83.4%), and β-pinene (1.5%) inhibited the growth of *P. acnes* and *S. epidermidis* at the lowest MIC values 0.31 and 2.5 µl/ml, respectively. Whereas, *Citrus natsudaidai* oil which was dominated by γ-terpinene (6.6%), limonene (81.6%), and β-myrcene (3.0%) exhibited against *P. acne* and *S. epidermidis* at MIC values 0.31 and 10 µl/ml, respectively.[30]

The chemical composition and efficacy evaluation of three Thai basil oils, including sweet basil, holy basil, and hoary basil against P. acnes have been also investigated. The results concluded that the sweet basil oil dominated by methyl chavicol (93.0%) and the major compounds of holy basil oil were eugenol (41.5%), γ-caryophyllene (23.7%), and methyl eugenol (11.8%).

Sweet basil and holy basil oils indicated that these could inhibit the growth of *P. acnes* with MIC 2.0 and 3.0% (v/v), respectively. Whereas, Hoary basil oil, contained high amount of geraniol (32.0%) and neral (27.2%) and small amounts of methyl chavicol (0.8%), did not show activity against *P. acnes* at the highest concentration tested (5.0% v/v).[31] In addition, the antibacterial activity against several bacterial strains of essential oils are also presented in Table 2.2

Table 2.2 Bacteria and their susceptibility to essential oils.[8]

Bacterial strains	MIC of various essential oils (µl/ml)[a]
Bacillus brevis	Eucalyptus 0.41, peppermint 1.66, cinnamon 200, basil 600
Bacillus cereus	Marjoram 0.4, oregano 0.4, thyme 400
Escherichia coli	Tea tree 2500, tansy 3900, oregano 250
Pseudomonas aeruginosa	Lemongrass 1.3, oregano 4000
Pseudomonas fluorescens	Cinnamon 1000, clove 1000, basil 20000, oregano marjoram 0.8
Salmonella typhi	Hyssop 6000, cinnamon 312, oregano 1250
Salmonella typhimurium	Lemongrass 1.66, palmarosa 0.80, eucalyptus 1.66, peppermint 0.80
Staphylococcus aureus	Tea tree 2500, cardamom 1600, lemongrass 0.3, eucalyptus 0.41, peppermint 1.66, oregano 500, thyme 1250
Staphylococcus epidermidis	Tea tree 6300, *Thymus longicaulis* 750, *Ocimum dictamnus* 300, *Stachys candia* 2000

a: In the references MIC values have been reported in the unit ppm, mg/ml, mg/l, %(v/v), µl/ml, and µg/ml. Each MIC values in Table 2.2 have been converted to µl/ml, whereby it was assumed that essential oils have the same density as water.

2.6 Determination of antibacterial activity [32]

2.6.1 Disc diffusion method

The disc diffusion method allows for the simultaneous testing of a large number of antibacterial in a relatively easy and flexible manner. In this method, the bacterial inoculum is adjusted to certain concentration, inoculated onto the entire surface of an agar plate with a sterilised cotton-tipped swab to form an even lawn. The sterilised paper disc (6 mm in diameter) impregnated with diluted antibiotic solution was placed on the surface of each agar plate using a sterilised pair of forceps. Then the plates were incubated aerobically and the diameter of zone inhibition was measured by a ruler or caliper. The bigger the diameter of the inhibition zone, the more susceptible is the microorganism to the antibacterial. The major disadvantages of this method are unable to generate the MIC value and difficult to examine the susceptibility of fastidious and slow-growing bacteria.

2.6.2 Agar dilution method

Agar dilution is a quantitative susceptibility testing method because MIC values can be obtained using the method. In this method, two-fold serial dilutions of an antibiotic made in agar medium and then bacterial suspensions were inoculated on the agar medium using a Cathra replicator with 1 mm pins. The advantages of agar dilution include the ability to simultaneously test the susceptibility of a number of bacteria in one plate and the ability to test susceptibility of fastidious organisms since the agar with supplements is able to adequately support the bacteria growth. Moreover, the test results yield MIC values for testing bacteria.

2.6.3 Broth micro-dilution method

Broth micro-dilution is another quantitative method routinely used in clinical laboratories. In this method, susceptibility panel in 96-well microtiter plates were containing various concentration of antimicrobial agents. Then, standardized numbers of bacteria was inoculated into the wells of 96-well microtiter and incubated overnight at 37^{o}C. The MIC value was observed as the lowest concentration where no viability was observed in the wells of 96-microwell plates after incubation. It is a widely utilized method, allowing for the simultaneous testing of multiple antimicrobials.

2.7 Plants used in this study

2.7.1 *Clausena harmandiana* (Pierre) [33]

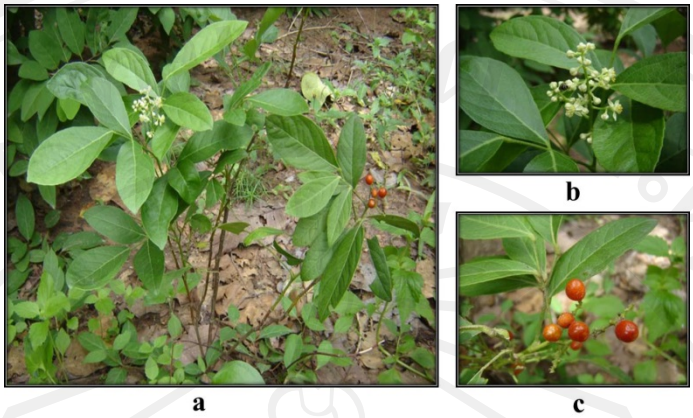


Figure 2.8 The shrub (a), flowers (b), and fruits (c) of *Clausena harmandiana*

Kingdom: Plantae
Order: Sapindales
Family: Rutaceae
Genus: *Clausena*
Species: *Clausena harmandiana*

C. harmandiana belongs to the family Rutaceae in Thailand commonly known as Song-fa or Pong-fa

Morphology: Perennial shrub, erect, growing to 39.35 to 66.91 cm, 4.9 to 7.7 mm in diameter, hairless stem, brownish green, leaves odd-pinnately, 3 to 7 leaflets, alternate ovate-oblong, acute at top, 6.81 to 11.05 cm long, 2.82 to 5.32 cm wide, dark green, lustrous, punctate, margins serrate with short hair, flowering during March-November, flowers terminal panicle, greenish yellow, yellow anther, 7.97 to 13.53 cm long inflorescens, produce fruit in April, fruit elliptic, green when young, whitish red when mature, mature fruit juicy with 1 seeded.

- **Benefit uses**

The young leaves and leaves are used as fodder for cattle and buffalo. The young leaves are also used as vegetable and medicinal plant. The roots have been known as health promoting herbs, and also frequently used for the treatment of the sickness, stomachache, and headache.[34]

2.7.2 *Clausena lansium* (Lour.) Skeels [35]

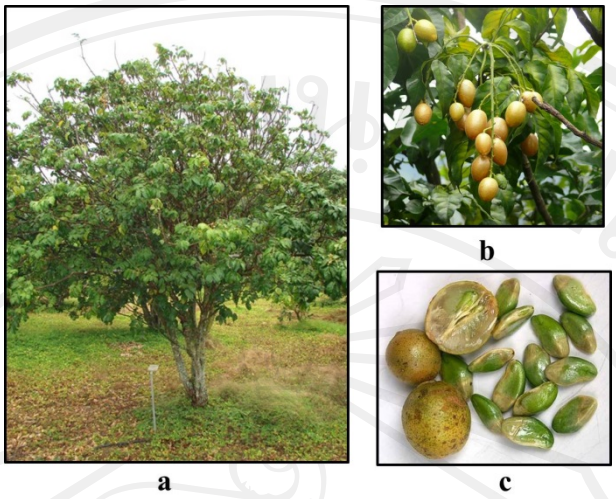


Figure 2.9 The tree (a), fruits (b) and seeds (c) of *Clausena lansium*

Kingdom:	Plantae
Order:	Sapindales
Family:	Rutaceae
Genus:	*Clausena*
Species:	*Clausena lansium*

C. lansium or wampee is a minor member of the family Rutaceae in Thailand commonly known as Ma-fai-jeen.

Morphology: Medium shrub, evergreen habit, growing up to 10 meters in height, flexible branches and gray-brown bark rough to the touch. The leaves are ruffled dark green consisting of 4 to 7 leaflets, elliptic or elliptic-ovate leaflets 7 to 10 cm long. The inflorescence is many branched panicle. It bears hundreds of small whitish or yellowish flowers. The number of fruit maturing on a panicle may vary from 1 to 30, hairy panicles 10 to 50 cm long. The fruits may be round or conical-oblong, up to 2.5 cm long. It contains 1 to 3 seeds which nearly fill the interior. The fruits are very juicy with a translucent pulp and can be eaten with the peel at a full ripe stage. The taste has pleasantly sweet, subacid, or sour depending on the variety and ripeness. There may be 1 to 5 oblong, thick seeds 1.25 to 1.6 cm long, bright-green with one brown tip.

- **Benefit uses** [36,37]

The fruits are very juicy with a translucent pulp that the taste has pleasantly sweet or sour. The leaves have been used as a folk medicine for the treatment of coughs, asthma and gastro-intestinal diseases, while the fruits are used for stomach upsets, digestive disorders, and coughs, and the seeds for gastro-intestinal diseases such as acute and chronic gastro-intestinal inflammation, and ulcers. In addition, Its roots are used to treat bronchitis and as an anti-malarial.

Previous studies on *C. harmandiana* and *C. lansium* have been investigated for chemical constituents and several compounds from the roots, seeds, and leaves have been reported. However, many researches have been mainly studied in the non-volatile compositions of their. The phytochemical investigations of *C. lansium* have revealed this plant contains carbazole alkaloids (clausenaline A, claulamine A, and claulamine B) from the stems and roots,[36,42] coumarins (8-hydroxypsoralen) from the peels,[37] and amides (lansiumamide B) from the seeds.[38] In addition, *C. harmandiana* concluded the characterization of carbazole alkaloids (Clausine H, Clausine L, Mukonidine, Clauraila E, Clauszoline-K, Heptaphylline) from the roots, [34,39,40] coumarins (clausmarin A, D, E and F) from the leaves[33], and phenylpropanoid derivatives (harmandianone, verimol B, (E)-methyl p-coumarate) from the fruits.[41] The biological activities of these components, especially carbazole alkaloids, have been presented antibacterial activity, antimalarial activity, anti-inflammatory activity, anti HIV-1 agents, antiplatelet aggregation, and antitumor promoting activity.[34]

The main chemical components of essential oil from various *Clausena* species, collected from different locations, are presented in Table 2.3. GC-MS analysis of the oils revealed the presence of monoterpenes, oxygenated monoterpenes and oxygenated sesquiterpenes were the major compounds. However, there are a few reports on the chemical composition of essential oil from *C. lansium* whereas *C. harmandiana* have not reported. In this study, the chemical compositions and anti-acne inducing bacterial activity of the essential oil from the leaves were investigated.

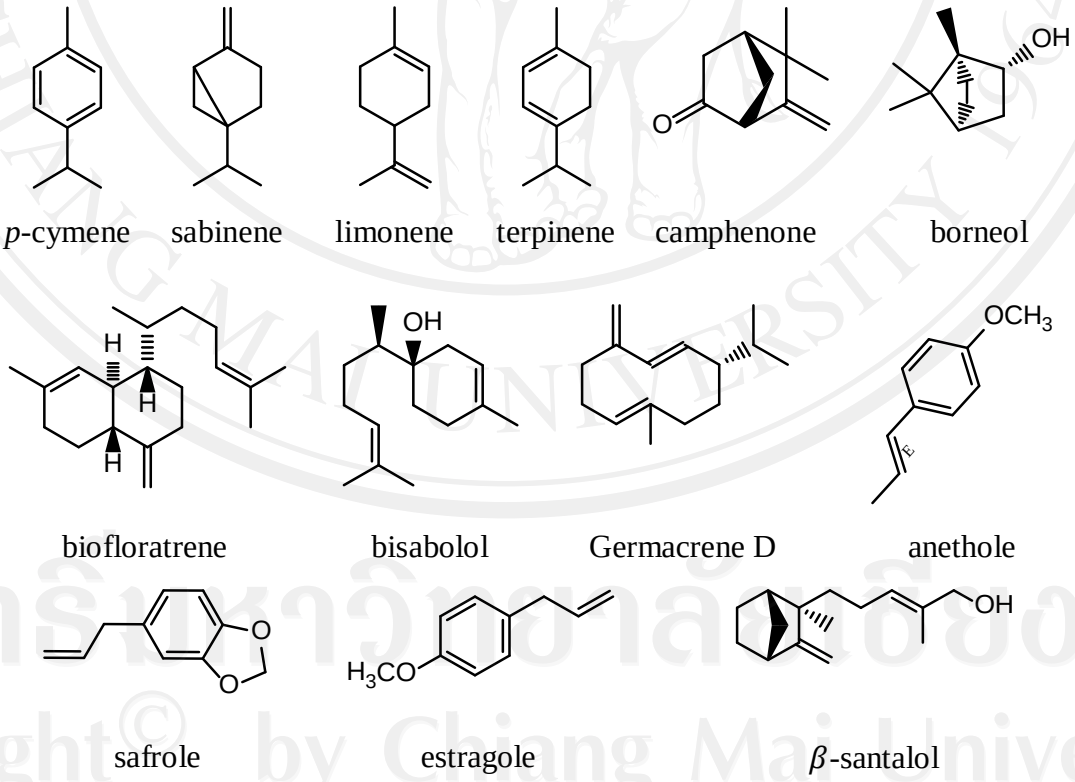
Table 2.3 Main chemical constituents of essential oil from various *Clausena* species

Species	part	Main chemical constituents of essential oil
C. dentata [43]	Leaf	Sabinene (21.27%), biofloratriene (19.61%), borneol (18.34%), *β*-bisabolol (17.68%)
C. excavata [44]	Leaf	Safrole (75.85%), terpinolene (17.86%), *3*-carene (2.68%)
	Twig	Safrole (65.80%), terpinolene (6.93%), *α*-pinene (8.85%), *p*-cymen-8-ol (4.39%), *p*-cymene (2.50%), *3*-carene (2.23%)
C. suffruticosa [45]	Leaf	Estragole (58.23%), anethole (33.20%), linalol (3.38%), *β* -ocimene (1.40%)
C. pentaphylla [46]	Leaf	Methyl eugenol (38.1%), sabinene (24.7%), *α*-terpinolene (13.8%), limonene (7.8%), safrole (6.7%)
C. anisata [47,48]	Leaf [47] (India)	*β*-pinene (32.80%), sabinene (28.30), germacrene-D (12.70%), estragole (6.40%), linalool (5.9%), limonene (1.2%)
	Leaf [48] (Nigeria)	Anethole (31.1%), trans-*β*-ocimene (20.0%), *β*-elemene (10.5%), estragole (6.9%), *α*-pinene (6.7%),
C. lansium [49]	Leaf	*β*-santalol (35.20%), bisabolol (13.70%), methyl santalol (6.9%), ledol (6.5%), sinensal (5.6%)
	Flower	*β*-santalol (50.60%), 9-octadecenamide (17.20%), sinensal (4.10%)
	Seed	phellandrene (54.8%), limonene (23.6%), *p*-menth-1-en-4-ol (7.5%)

In addition, the essential oil from the leaves of *C. dentata* [43] and *C. anisata* [47] have been shown larvicidal activities. The essential oil of C. dentata exhibited against *Aedes aegypti* larvae with LC_{50} and LC_{90} values of 140.2 and 341.6 mg/l, respectively. While, the oil of *C. anisata* presented against three mosquito species as *Culex quinquefasciatus, Aedes aegypti* and *Anopheles Stephensi* larvae with LC_{50} values 140.96, 130.19 and 119.59 mg/l, respectively.

Moreover, the essential oil of *C. suffruticosa*[45] leaves have been shown antimicrobial activities. The oil could inhibit the growth of Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Bacillus polymyxa* and *Bacillus megaterium*) and Gram- negative bacteria (*Salmonella typhi, Shigella flexneri, Proteus mirabilis* and *Escherichia coli*) with the range of minimum inhibitory concentration (MIC) from 25-100 µl/ml.

Some structures of the major chemical constituents of essential oil from genus *Clausena* are shown in Figure 2.10.



p-cymene sabinene limonene terpinene camphenone borneol

biofloratrene bisabolol Germacrene D anethole

safrole estragole *β*-santalol

Figure 2.10 Structure of the major compounds of essential oil from *Clausena*

2.7.3 *Elsholtzia communis* (Collett & Hemsl.) Diels [50,51]

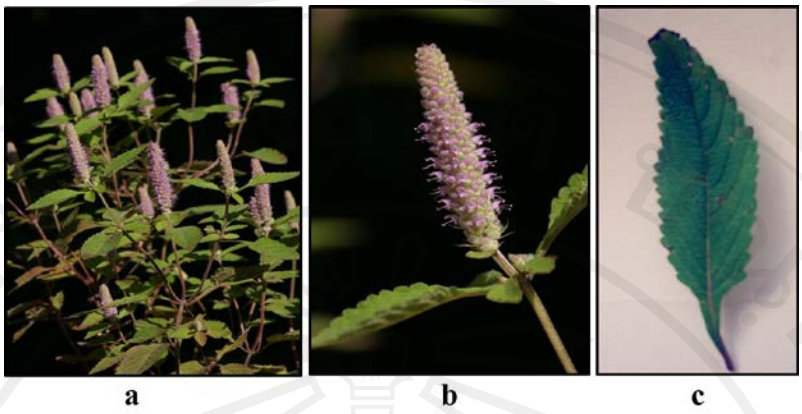


Figure 2.11 The shrub (a), flower (b), and leaf (c) of *Elsholtzia communis*

Kingdom: Plantae
Order: Lamiales
Family: Lamiaceae
Genus: *Elsholtzia*
Species: *Elsholtzia communis*

E. communis belongs to the Lamiaceae family in Thailand commonly known as e-luen.

Distribution: China, Myanmar, Thailand

Thailand: Mae Hong Son, Chiang Mai

Morphology: Herbs 60 cm tall, with strong citral fragrance. Stems erect, purple-red, densely retrorse white pubescent, much branched at base. Petiole 2 to 5 mm, densely white pubescent; leaf blade ovate to oblong, herbaceous, adaxially white villous, abaxially pubescent, yellowish glandular, margin serrate. Spikes terminal, cylindric, 1 to 4.5 x 0.8 to 1 cm, com-pact; verticillasters numerous; rachis densely white villous; bracts linear, to 3.5 mm, densely white pilose. Pedicel ca. 1 mm, densely white villous. Calyx tubular, to 4 mm in fruit, apex recurved, densely gray lanate-villous outside; teeth subequal, slightly closed in fruit. Corolla funnelform, ca. 3 mm, pilose, glandular outside, obscurely hairy annulate inside; upper lip oblong, emarginate, ciliate; lateral lobes of lower lip less than 1/2 as wide as middle lobe. Style apex unequally 2-cleft. Nutlets oblong, ca. 0.7 mm, sparsely brown hairy. Flowering and fruiting in October to December.

2.7.4 *Elsholtzia stachyodes* (Link) C.Y.Wu [50,51]

Figure 2.12 The shrub (a), flower (b), and leaf (c) of *Elsholtzia stachyodes*

Kingdom: Plantae
Order: Lamiales
Family: Lamiaceae
Genus: *Elsholtzia*
Species: *Elsholtzia stachyodes*

E. stachyodes belongs to the Lamiaceae family in Thailand commonly known as harn.

Distribution: India, Nepal, China, Myanmar, Thailand

Thailand: Mae Hong Son, Chiang Mai, Prachuap Khiri Khan

Morphology: Herbs 30 to 100 cm tall. Stems erect, yellow-brown or purplish, sparsely white floccose-pubescent, ± glabrescent, much branched. Petiole 0.5 to 4 cm, nearly as long as blades, white, adaxially puberulent; leaf blade rhombic - ovate, 2.5 to 6 x 1.5 to 3.5 cm, thin papery, adaxially sparsely pubescent, abaxially with sparse yellowish glands, pubescent on veins, base cuneate to broadly cuneate, decurrent, margin incised-serrate above base, apex abruptly acuminate. Spikes terminal and axillary, terminal ones 4 to 8.5 cm, ± interrupted; verticillasters few flowered; bracts subulate-linear, longer than corolla. Pedicel ca. 0.5 mm. Calyx campanulate, ca. 1.5 mm, densely white villous outside; teeth lanceolate, subequal, pubescent inside; fruiting calyx slightly dilated, tubular-campanulate, ca. 2 mm.

Corolla white, sometimes purple-red, ca. 2x as long as calyx, pubescent outside, glabrous inside, tube funnelform, upper lip emarginate, middle lobe of lower lip elliptic, lateral lobes rounded. Anterior stamens undeveloped, posterior 2 included or slightly exserted. Nutlets yellowish, ellipsoid. Flowering and fruiting in September to December.

2.7.5 *Elsholtzia griffithii* Hook.f [51]

Figure 2.13 The shrub (a), flower (b), and leaf (c) of *Elsholtzia griffithii*

Kingdom:	Plantae
Order:	Lamiales
Family:	Lamiaceae
Genus:	*Elsholtzia*
Species:	*Elsholtzia griffithii*

E. griffithii belongs to the Lamiaceae family in Thailand commonly known as loom-poom.

Distribution: Myanmar, Thailand

Thailand: Mae Hong Son

Morphology: similar to *Elsholtzia stachyodes* (Link) C.Y.Wu but differs in having rhomboid-ovate leaves and longer petioles. Flowering and fruiting in September to December.

2.7.6 *Elsholtzia* sp.

a b

Figure 2.14 The flower (a), and leaf (b) of *Elsholtzia* sp.

Kingdom: Plantae
Order: Lamiales
Family: Lamiaceae
Genus: *Elsholtzia*

E. sp. belongs to the Lamiaceae family in Thailand commonly known as e-luen-pa.

Distribution: Thailand (Chiang Mai)

Elsholtzia species is composed of essential oil. The chemical constituents of essential oil from various *Elsholtzia* species, collected from different locations were reported.[9] The main chemical compounds are presented in Table 2.4. The dominant compounds were acylfurans derivatives, monoterpenes and monoterpenoids as the biochemical markers of the oil. Some structures of the major chemical constituents of essential oil from genus *Elsholtzia* are shown in Figure 2.15.

The essential oil from some *Elsholtzia* species have been shown antibacterial activity against some bacteria frequently result in respiratory infections in human, such as *Staphylococcus aureus*, *Bacillus typhi*, *Aeruginosus bacillus*, and *Diplococcus intracellularis.*[9]

Table 2.4 Main chemical constituents of essential oil from various *Elsholtzia* species [9]

Species	Main chemical constituents of essential oil
E. rugusola	Thymol, carvacrol, 1,8-cineole, linalool, camphor, *β*-dehydroelsholtzia ketone, elsholtzia ketone
E. argyi	Limonene, geranial, neral, *β*-trans-ocimene
E. ciliata	*β*-dehydroelsholtzia ketone, elsholtzia ketone, 2-methyl-1, 3,5-trimethyl-benzene, aromadendrene, d-carvone, limonene
E. patrini	*β*-dehydroelsholtzia ketone, elsholtzia ketone, d-carvone, aromadendrene, 2-methyl-1, 3,5-trimethyl-benzene, limonene
E. kachinensis	*β*-dehydroelsholtzia ketone, carvone, octenyl acetate
E. densa	Ocimene, 1-p-menthadien-1, *β*-cubebene, thymol

In 2008,[52] the essential oil from the stem of flowers of *E. splendens* was extracted by steam distillation and the chemical compounds were analyzed by GC and GC-MS. Nine compounds were identified, amounting for 93.00% of the oil with dehydroelsholtzia ketone (82.46%) and elsholtzia ketone (5.96%) were the main compounds. The antibacterial activity against *P. acnes* of this oil showed at MIC 0.31 µl/ml.

In 2013,[53] essential oil from the leaves, flowers and seeds of *E. ciliata*, which monoterpenoids and sesquiterpenoids were the main compounds could inhibit the growth of *Bacillus subtillis*, *Escherichia coli*, *Salmonella enteritidis*, *Shigella flexneri*, *Salmonella typhi*, *Staphylococcus aureus* (the six bacterial strains) and *Candida albicans* (the yeast strain). The oil obtained from the leaves has been shown higher inhibitory activities especially against *B. subtillis* and *E. coli* with minimum inhibitory concentration (MIC) 0.02 and 1.08 µl/ml, respectively.

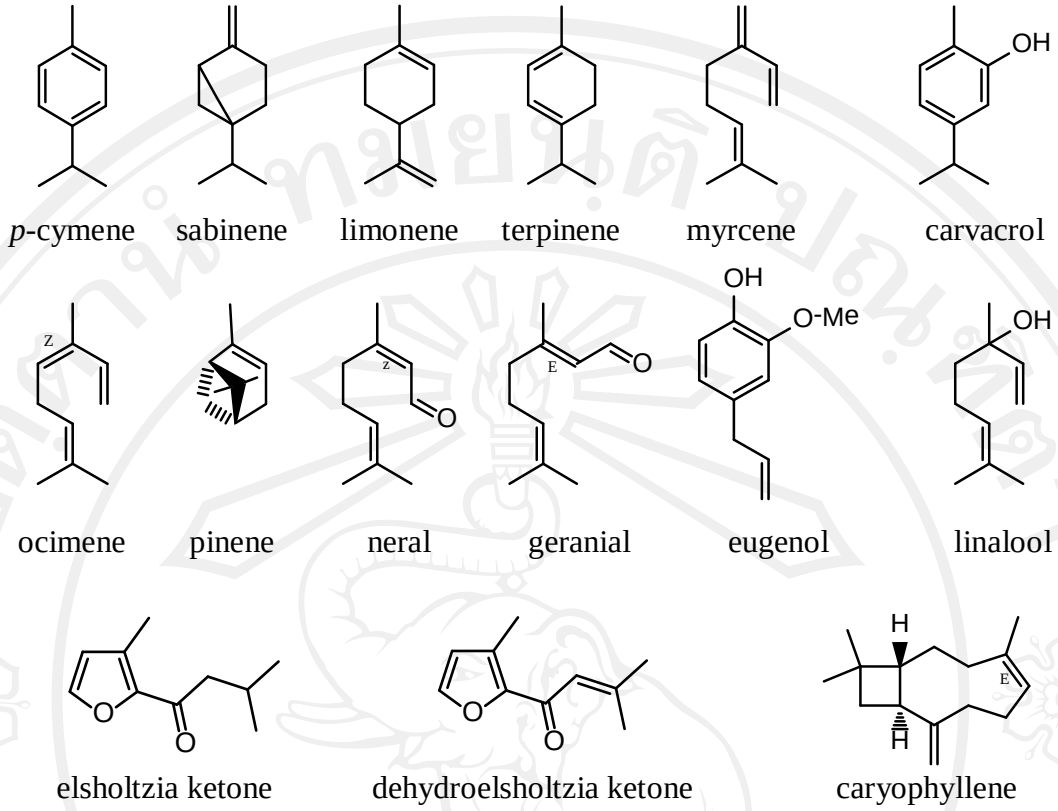


Figure 2.15 Structure of the major compounds of essential oil from *Elsholtzia*